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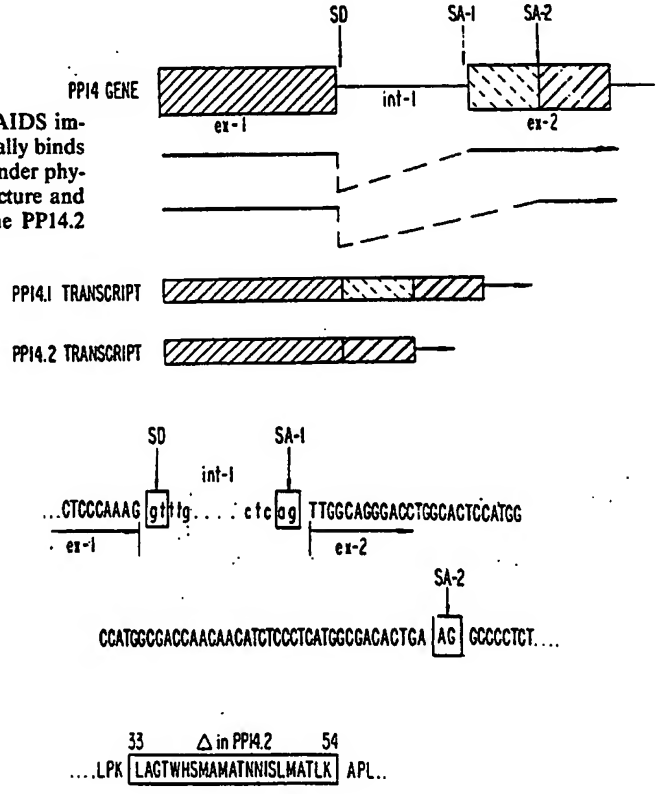
(54) Title: PP14-BASED THERAPY

(57) Abstract

Method for treating a patient suffering form non-AIDS immunosuppression by administering a reagent that specifically binds to an isoform of PP14, or a receptor for a PP14 isoform under physiological conditions. A schematic of the PP14 gene structure and partial sequence information for the PP14 genes and the PP14.2 protein are shown in the figure.



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DESCRIPTION

PP14-Based Therapy

Background of the Invention

This invention relates to placental protein 14 ("PP14") and monoclonal antibodies thereto, and their uses.

Human PP14 is a 28 kilodalton glycoprotein expressed during the first and second trimesters of pregnancy by the endometrium. During this period, it constitutes up to 5-10% of the total secreted protein from the endometrial decidua (Julkunen et al., 92 Br. J. Obstet. Gynaecol. 1145, 1985). PP14 accumulates to significant levels in serum from pregnant women as well. In addition, PP14 is found at high concentrations in male seminal fluid (19-515 mg/l) (Pockley et al., 43 Biochem. Soc. Trans. 317, 1989), although the cellular source of PP14 in the male reproductive tract has not been determined.

PP14 belongs to a class of proteins called β-lactoglobulins, which include lactoferrin (Rado et al., 64 Blood 1103, 1984) and the retinoic acid receptor (Papiz et al., 324 Nature 383, 1986). A PP14 cDNA has been cloned from endometrium, and analysis of this endometrial PP14 cDNA has shown the PP14-coding region to be approximately 70% homologous to other β-lactoglobulin family members, and their genomes are similar in size and organization (Vaisse et al., 9 DNA Cell. Biol 401, 1990).

PP14 has been shown to inhibit lymphocyte proliferation (Bolton et al., in Lancet 593, 1987). Extracts of human decidual tissue were added to mixed lymphocyte cultures (MLCs), and a linear relationship was observed between the quantity of PP14 present, and the inhibition of lymphocyte proliferation observed. Id. Moreover, an anti-PP14 monoclonal antibody added to the MLCs inhibited the anti-proliferative effect, indicating a functional link between PP14 and anti-proliferative activity. Subse-

quent studies have noted an inhibitory effect of PP14 on the synthesis of IL-1, IL-2 and soluble IL-2 receptors by peripheral blood mononuclear cells (Pockley et al., 16 Biochem. Soc. Trans. 794, 1988; Pockley et al., 77 Clin. Exp. Immunol. 252, 1989; and Pockley & Bolton, 69 Immunology 277, 1990). Okamoto et al., 26 Amer. J. Reprod. Immunol. 137, 1991 indicate that PP14 suppresses natural killer cell activity.

It has been proposed that PP14 be used for non-specific immunosuppression in patients in need of such immunosuppression, such as those suffering from autoimmune conditions, inflammatory conditions and allergic conditions. Bolton et al., U.S. Patent 5,039,521. Specifically, Bolton et al. propose use of PP14 to treat arthritis, rheumatoid arthritis, asthma, graft-versus-host disease, organ rejection, osteoarthritis, systemic lupus erythematosus, atopic allergy, multiple sclerosis, allergic dermatitis, inflammatory bowel disease, psoriasis, sarcoidosis, and other inflammatory disorders. In addition, they suggest treatment of a lymphoproliferative disorder, such as malignant non-Hodgkin's lymphoma, Hodgkin's disease, or malignant histiocystotis. Suggestion is also made to treat inflammatory and autoimmune diseases, and to treat infertility and a neoplastic disorder such as leukemia. Finally, it is stated that monoclonal antibodies to PP14 can be used to treat an immune system disorder, specifically, acquired immunodeficiency syndrome (AIDS).

Summary of the Invention

Applicant has discovered that PP14 is expressed in cells outside of the female and male reproductive tracts, and specifically, that PP14-encoding mRNA and PP14 protein is expressed in hematopoietic cells. In particular, when the human myelogenous leukemia cell line K562 (which has bipotential differentiative capacities, as it can be chemically induced to differentiate along erythroid and mega-

karyocytic lineages) is caused to differentiate along the megakaryocytic lineage, PP14 cDNAs are detectable.

This hematopoietically expressed PP14 has two polypeptide isoforms, encoded by two mRNA species that arise through alternative splicing of the PP14 gene. These two polypeptide isoforms, and their corresponding mRNAs, are designated "PP14.1" and "PP14.2" herein. Previous studies of PP14 in the female and male reproductive tracts (discussed above), recognized only one PP14 isoform, corresponding to PP14.1.

The unexpected finding of PP14 in cells of the megakaryocytic lineage, and particularly in the end-cell of that lineage, the platelet, indicates the use of PP14 in otherwise unexpected areas. These discoveries are evidence of PP14's pathophysiological role. Specifically, the release of PP14 from platelets in the course of platelet disorders, including coagulopathies (such as disseminated intravascular coagulation), will lead to generalized immunosuppression in patients suffering from such disorders, with all of PP14's attendant untoward (immunosuppressive) effects. Thus, Applicant has discovered that it is important to reduce the effect that PP14 release from platelets has on pathogenesis. This indicates that, for a variety of disease conditions, PP14 blockade, as opposed to potentiation, is the desired therapeutic endpoint for certain clinical conditions. To this end, Applicant discloses several methods for treatment of patients suffering from untoward effects of platelets, and for blocking immunosuppression associated with PP14 release from platelets in such patients. These methods are based upon blocking PP14 effects. This comprises, but is not limited to, the therapeutic use of anti-PP14 antibodies, peptides which block PP14 activity, solubilized PP14 receptor, and anti-PP14 receptor antibodies, or their equivalent (e.g., active fragments thereof, which can be identified by standard procedures). Compositions or

therapeutic formulations, including these therapeutic agents are also disclosed.

The invention also includes administration, to a patient suffering from platelet-induced immunosuppression, of a reagent that will inhibit PP14 production by hematopoietic cells of the patient. Antisense PP14 oligonucleotides and ribozymes are suitable reagents for interfering with PP14 transcription and/or translation. Methods for identifying additional reagents for blocking PP14 production at the mRNA or protein levels are straightforward, and readily practiced by those familiar with the art.

Specifically, the invention features methods for treating a patient suffering from leukemia, where the leukemic cells of the patient have megakaryocytic differentiative potential and produce a PP14 polypeptide with immunosuppressive activity. The methods for blocking PP14 function and reducing PP14 production cited above can also be applied in this clinical setting.

The invention also features production of anti-PP14.1 and PP14.2-specific antibodies and PP14 receptors for therapeutic and diagnostic applications. The discovery of a second PP14 isoform in hematopoietic cells permits the generation of well-characterized anti-PP14 monoclonal antibodies with specificity for one or both isoforms. Methods for producing both anti-PP14.1-specific and anti-PP14.2-specific antibodies are disclosed, and make use of the precise determination in the present invention of the amino acid sequence difference between the two isoforms. This knowledge permits the production and use as immunogens of peptides corresponding to junctional and internal amino acid sequences that distinguish between PP14.1 and PP14.2 polypeptides.

Such isoform-specific anti-PP14 antibodies are useful for detection of PP14 isoforms in the serum of patients, to determine which patients can benefit from PP14 blockade therapy, and to monitor therapeutic responses to such therapy. In addition, PP14 isoforms can serve as a marker

for certain platelet disorders, such as disseminated intravascular coagulation, and PP14 isoform-specific or non-specific diagnostic assays are useful in this clinical setting. In pregnant women, PP14-isoform-specific antibodies allow for the discriminative analysis of hematopoietic cell- and endometrial cell-derived PP14 polypeptides.

This invention also allows production and use of a polypeptide derivative of a PP14 receptor (and/or antibodies with specificity for such a receptor) for blocking a functional interaction between a PP14 polypeptide and its receptor. Methods for cloning receptors for known ligands, such as ones based upon the use of readily purified ligand:immunoglobulin Fc conjugates, are well-established and can be expeditiously carried out by those familiar with the art.

Thus, this invention features any method by which the activity of either PP14 polypeptide isoform is reduced to thereby interfere with PP14-induced immunosuppression. Such reduction of PP14 activity is useful for treatment of disease states (other than AIDS) characterized by elevated PP14 levels. Those in the art will recognize that all of the various methods discussed above, as well as others (such as use of blocking PP14 peptides or blocking anti-idiotypic antibody mimics of PP14), achieve the same end as one another, that is, the prevention of PP14-induced immunosuppression, by in some way affecting the end-function or production of PP14 in the body.

The invention also features therapeutic methods targeted at various immunological diseases. Unlike diseases that are the subject of PP14 blockade therapy, in which there is a need for reversal of immunosuppression, these other immunological diseases require exogenous immunosuppressive agents. The PP14.2 polypeptide isoform, according to the present invention, provides a distinct immunosuppressive agent for pharmaceutical use which can be used independently of (or jointly with) PP14.1, or other immu-

nosuppressive polypeptides. PP14 therapeutic preparations having the hematopoietic PP14.2 polypeptide have advantages over PP14 therapeutic preparations consisting exclusively of the PP14.1 polypeptide, with respect to both stability (in blood and other tissue fluids) and therapeutic efficacy for diseases described by Bolton, supra. Thus, PP14 polypeptides can be used in known therapeutic methods which target cytokine circuits in the immune system, such as those predicated upon soluble interleukin-1 receptors for blocking interleukin-1:interleukin-1 receptor interaction, for treating acute inflammatory conditions. PP14.2 can also be used to suppress natural killer cell function.

The invention also features use of hematopoietic cells as a source of PP14 with immunosuppressive activity, providing the possibility of isolating both PP14.1 and PP14.2 polypeptides concurrently. Furthermore, hematopoietic cells, as well as other cells, can be used as cellular transfection targets for the production of recombinant PP14.1 and PP14.2 polypeptides which will process and secrete the desired PP14 isoform in an appropriate fashion. Both intact versions of these polypeptides, as well as polypeptide derivatives of these polypeptides having a desired biological activity, can be readily generated. A preferred composition includes a PP14 polypeptide sequence linked to a glycophosphatidylinositol (GPI) moiety. This membrane-anchored PP14 variant is expressed at the cell surface and can be preferentially cleaved from the surface by virtue of the GPI membrane anchor.

The invention also concerns production of PP14.1:PP14.2 heterodimers and PP14.2 homodimers. Dimers comprising PP14.2 polypeptide are more stable than PP14.1:PP14.1 homodimers, and provide a more optimal therapeutic and diagnostic reagent. Standard cotransfection strategies can be effectively used to produce the heterodimers.

Thus, in a first aspect, the invention features a method for treating a patient suffering from a non-AIDS

immunosuppression condition, by administering to the patient a reagent that specifically binds to at least one isoform of PP14, or to a receptor for a PP14 isoform, under physiological conditions, to thereby prevent or reduce binding of PP14 to its receptor.

By "non-AIDS immunosuppression" is meant a condition which is caused by (or adversely affected by, or related to) an elevated level of a PP14 isoform. Provision of the reagent will allow the reagent to bind to the PP14 isoform(s), or its receptors, and thus neutralize the PP14 activity. Examples of such conditions include platelet disorders, such as disseminated intravascular coagulation, platelet-induced immunosuppression secondary to platelet transfusion, and thrombocytosis, and leukemia.

In preferred embodiments, the reagent comprises, consists of, or consists essentially of, a compound selected from an antibody specific for PP14.1; an antibody (monoclonal or polyclonal, which may be a humanized murine or non-primate antibody) specific for PP14.2, and antibody specific for PP14.1 and PP14.2, a receptor for a PP14 isoform, a portion of a receptor for a PP14 isoform, an antibody to a PP14 receptor, a polypeptide portion of a PP14 isoform, or any other reagent which competitively inhibits binding of PP14 to a PP14 receptor in vivo or in vitro, e.g., a PP14 receptor antibody or anti-idiotypic antibody. Such antibodies may be humanized, that is the framework may be derived from a human antibody and the complementarily determining regions from another organism, e.g., a mouse or non-human primate. The reagent preferably competitively inhibits binding of PP14 and its receptor.

In one example, the polypeptide portion of PP14 includes that portion of the polypeptide defined by amino acids 33 through 54 of PP14.1 (i.e., specific to PP14.1 and not PP14.2); the reagent may be (a) an anti-idiotypic antibody mimic of PP14 which competes with PP14 for binding to (but does not activate) a cellular receptor for

PP14; (b) a soluble polypeptide derivative of a receptor for PP14 having the extracellular domain of a PP14 receptor free of the receptor's native transmembrane and cytoplasmic domains; or (c) a soluble polypeptide derivative of a PP14 receptor:immunoglobulin Fc (or other useful targeting polypeptide) chimeric polypeptide.

In a second aspect, the invention features a method for cloning a PP14 receptor, by determining an amino acid sequence of the receptor and screening a library for a clone of the receptor using an oligonucleotide probe corresponding to the amino acid sequence.

In a preferred embodiment, the method is a method for cloning a complementary DNA corresponding to a PP14 receptor including providing a chimeric polypeptide having a PP14 polypeptide linked to the sequence of an Fc region of an immunoglobulin (or its equivalent); contacting the chimeric polypeptide with a cellular extract from a cell expressing a PP14 receptor under conditions suitable for forming a complex of the chimeric polypeptide bound to the PP14 receptor; precipitating complex by contacting protein A or protein G (or its equivalent) conjugated to an insoluble matrix with the complex; recovering the PP14 receptor from the matrix; determining the amino acid sequence of a portion of the PP14 receptor; and screening a cDNA library for a PP14 receptor CDNA using an oligonucleotide probe corresponding to the amino acid sequence of the PP14 receptor.

In a third aspect, the invention features a method for producing an antibody with specificity for a receptor for a PP14 polypeptide, by immunizing a host with a portion of a receptor for a PP14 polypeptide.

In a fourth aspect, the invention features a method for blocking immunosuppression in a patient by administering to the patient a reagent that blocks transcription of a PP14 gene and/or translation of a PP14 transcript.

In preferred embodiments, an antisense oligonucleotide, ribozyme, or a triplex-forming nucleic acid is used

to block transcription and/or translation of a PP14 polypeptide.

In a fifth aspect, the invention features a method for identifying a reagent that blocks transcription of PP14, by screening a chosen compound for its capacity to block a PP14 isoform promoter-driven transcription of a reporter gene.

In a sixth aspect, the invention features a method for identifying a patient with a platelet disorder, by contacting a sample from the patient with an antibody or other reagent with specificity for PP14.1 and/or PP14.2, and determining the amount of reaction of the antibody with the sample, compared to the amount of reaction observed in a normal patient not having a disorder.

In a seventh aspect, the invention features a method for preparing PP14.1-specific antibodies, by immunizing a host with a polypeptide having an antigenic portion of the polypeptide defined by amino acids 33 to 54 of PP14.1.

In related aspects, the invention features a method for preparing PP14.2-specific antibodies, by immunizing a host with a polypeptide having a sequence of amino acids overlapping the junctional site of amino acids 32-33 of PP14.2; a pharmaceutical or other composition including an antibody specific for PP14.1 or PP14.2 in a pharmaceutically acceptable buffer; a pharmaceutical or other composition, including a portion of a PP14 polypeptide which competitively inhibits the binding of a native PP14 polypeptide to its receptor in a pharmaceutically acceptable buffer; a pharmaceutical or other composition, including an anti-idiotypic antibody mimic of PP14 which competes for binding to, but does not activate, a cellular receptor for PP14; a pharmaceutical or other composition including a soluble polypeptide derivative of a receptor for PP14 having the extracellular domain of a PP14 receptor free of the receptor's native transmembrane and cytoplasmic domains; and a pharmaceutical or other composition including a PP14-binding portion of an extracellular domain of

a receptor for PP14 linked to a Fc domain of an immunoglobulin heavy chain.

By "pharmaceutical" is meant a composition containing as its active reagent at least the noted compound in a buffer which is suitable for administration to a human or other animal. The term is used in its art-recognized manner, and includes those standard reagents known in the art.

In another related aspect, the invention features a method for treating a patient in need of immunosuppression by administering to the patient a PP14.2 polypeptide.

In preferred embodiments, the patient suffers from an autoimmune disease, rheumatoid arthritis, an allergic disorder, transplant rejection, or graft-versus-host disease.

In another aspect, the invention features a method for preparing a PP14 polypeptide by isolating PP14 from a hematopoietic cell, e.g., a phorbol myristate acetate-induced cell, or a platelet; and the invention features the resulting purified PP14 polypeptide PP14.1, or PP14.2.

In related aspects, the invention features a method for preparing a PP14.2 polypeptide by introducing a transcriptional cassette having a promoter transcriptionally linked to a portion of a coding sequence for PP14.2; a composition having a transcriptional cassette having a eukaryotic or prokaryotic promoter linked to a portion of a coding sequence for PP14.2; and a composition including a PP14 receptor-binding portion of a PP14 polypeptide linked to a glycosylphosphatidylinositol (GPI) moiety or its equivalent cell surface binding portion.

In still another related aspect, the invention features a method for producing a PP14 polypeptide, by transfecting a cell with a transcriptional cassette including nucleic acid encoding a portion of a PP14 polypeptide linked to a cell targeting signal sequence (e.g., glycosylphosphatidylinositol (GPI)), to express a PP14 polypeptide linked to the signal sequence at the cell surface; and cleaving the signal sequence with a cleaving reagent,

thereby releasing the PP14 polypeptide into the medium for subsequent recovery.

The invention also features a composition having a portion of a PP14 receptor-binding polypeptide linked to a portion of a member of a specific-binding pair (e.g., streptavidin or avidin with a biotin-binding domain); and a purified or recombinant PP14 receptor, PP14 receptor antibody, anti-idiotypic antibody, and purified nucleic acids encoding therefore; and a cell having a transcriptional cassette including nucleic acid encoding a portion of a PP14 polypeptide linked to a cell surface resident specific signal sequence, such as a glycosylphosphatidylinositol (GPI) signal sequence, to express a PP14 polypeptide linked to a GPI moiety at the cell surface.

By "purified" is meant that the composition is different from that naturally occurring in nature in that the active ingredient is at higher purity relative to one or more other compounds naturally associated with it. By "recombinant" is meant produced by genetic engineering procedures, e.g., it is expressed from a cloned gene or its equivalent.

The invention also features a composition including a heteromultimer of PP14.1 and PP14.2 polypeptide chains, or a homomultimer of PP14.2 polypeptide chains, and a method for producing such a heteromultimer by cotransfecting PP14.1 and PP14.2 transcriptional cassettes into a cell.

In other aspects, the invention features a method for suppressing natural killer cell activity by contacting such cells with a PP14.2 polypeptide; a PP14 polypeptide modified to target a cell surface (e.g., an antigen-presenting cell), e.g., by covalent bonding of a polypeptide moiety, such as GPI or its equivalent; and a method for coating an antigen-presenting cell by use of such a targeted PP14 polypeptide. Specifically, a PP14-GPI chimeric polypeptide will target the cell surface of an antigen-presenting cell, or a PP14-streptavidin polypeptide

will target a cell coated with biotin. Such cells are useful for therapeutic or diagnostic procedures based upon detection of or binding to PP14, as discussed herein.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments of the invention, and from the claims.

Description of the Preferred Embodiments

The drawings will first briefly be described.

Drawings

Fig. 1 is a RNA transfer blot demonstrating that PP14 mRNA expression is induction- and lineage-specific in leukemic cells differentiating along the megakaryocytic lineage. Total RNA from uninduced and induced K562, HL-60, U937, and PLB-985 cell lines were probed with a purified PP14 probe. K562 cells were induced along the megakaryocytic lineage for 0, 24, 48, and 72h with PMA (10nM), or along the erythroid lineage for 0, 24, 48, and 72h with hemin (50μM). HL-60 cells were induced along the macrophage lineage for 0 or 48h with PMA (10nM), or along the neutrophil lineage for 0 or 48h with DMSO (2%). U937 cells were induced along the macrophage lineage for 0 or 48h with PMA (10nM), and PLB-985 were also induced along the macrophage lineage for 0 or 48h with PMA (10nM). Peripheral blood mononuclear cells (PBL) stimulated with anti-CD3 antibody for 3 days serves as a negative control. The approximately 800bp band corresponding to PP14 mRNA is indicated by an arrowhead.

Fig. 2 is a RNA transfer blot demonstrating the kinetics of PP14 expression after PMA-induction and showing that PP14 mRNA is detectable after 6 hours of inducer treatment. Total RNA from K562 cells induced with PMA (10nM) for 0, 0.5, 1, 2, 3, 6, 12, or 24 hours was isolated and hybridized to a purified PP14 probe.

Fig. 3 is a copy of an autoradiogram showing PP14 immunoprecipitates from PMA-induced K562 cultured medium.

K562 cells were metabolically labeled by culturing in cysteine and methionine free-medium with the addition of ^{35}S-cysteine and ^{35}S-methionine for 24 or 48h. Cells were removed and supernatants were immunoprecipitated with protein G and an anti-PP14 polyclonal or monoclonal antibody (Ab). Bound product was run under reducing conditions on a 12% PAGE-SDS gel. The expected mobility of PP14.1 and PP14.2 are noted. Upon longer exposure, a 28kD band (representing PP14.1) is visible in the 24 hour lane as well.

Fig. 4 is a two-way mixed lymphocyte culture showing that a potent inhibitory effect of PMA-induced K562 conditioned medium upon alloantigen-stimulated proliferation can be neutralized with anti-PP14 antibodies. K562 cells were induced with PMA (10nM) for 24 or 48h to induce PP14 expression. Conditioned medium was added to the mixed lymphocyte culture (MLC) at 50% v/v ratio with or without a rabbit polyclonal anti-PP14 antiserum (1:100 dilution). The MLC was harvested at days 5, 6, or 7, and incorporated ^{3}H-thymidine cpm were counted. Values represent the average of triplicate cultures, and the experiment was repeated two times with similar results. No effect on the observed inhibition by conditioned medium was seen with normal rabbit serum or anti-TGFβ Ab (data not shown).

Figs. 5a-5c show a schematic representation of PP14.1 and PP14.2 mRNAs and highlight the nucleotide and amino acid sequence differences between the two. Fig. 5a: Exon-1 and exon-2, along with intron-1, of the PP14 gene are schematized, with differential shading of the two halves of exon-2; the upstream portion of exon-2 is eliminated from PP14.2 mRNA by alternative splicing. The dashed line indicates the stretch of sequence deleted by such alternative splicing. Fig. 5b: The nucleotide sequence of the portion of the PP14 gene involved in the alternative splicing event is shown. The common splice donor (SD) site and the two alternative splice acceptor (SA) sites are highlighted by arrows, and the dinucleo-

tides at the splice sites are boxed. Fig. 5c: The amino acid sequence of the portion of PP14.1 that is deleted in the PP14.2 polypeptide is boxed. Numbers indicate the boundaries of the deletion, based upon numbering of the PP14.1 amino acid sequence.

Fig. 6 is a reverse transcriptase-polymerase chain reaction (RT-PCR) analysis using PP14-specific primers able to distinguish between the PP14.1 and PP14.2 mRNA species in PMA-induced K562 cells of the megakaryocytic lineage and placenta cells. PP14.2 mRNA is present in significant amounts only in K562 cells, not in placenta cells taken from 18, 28, 36 and 37 weeks old placenta. Molecular weight numbers are included on the right-hand side.

Fig. 7 is a RT-PCR analysis using PP14-specific primers showing that PP14 expression is restricted to tissues of the hematopoietic and reproductive systems, and that whereas both PP14.1 and PP14.2 isoforms are expressed at significant, and apparently equimolar, levels in hematopoietic cells, the PP14.1 isoform. 2.5μg total RNA was reverse transcribed using a 3'-end specific primer for PP14. The resulting product was used directly in a PCR reaction with a 5'-end specific primer for PP14. Lane 1: uninduced K562 cells. Lanes 2 and 3: PMA-induced K562 cells at 24 and 48h, respectively. Lane 4: brain. Lane 5: spleen. Lane 6: small intestine. Lane7: liver. Lane 8: kidney. Lane 9: brain meninges. Lane 10: KM-102 bone marrow fibroblastoid stromal cells. Lane 11: placenta at 18 weeks. Lane 12: placenta at term. Lane 13: fibroblasts. Lane 14: primers alone. Lane M - molecular weight markers. Arrows indicate the relative portions of Pp14.1 and PP14.2 as well as molecular weight markers.

Platelet-derived PP14 Isoforms and Dimers

Placental protein 14 (PP14), originally named after the placental tissue it was thought to derive from, was

later, shown to originate from associated endometrial tissue (Vaisse et al., 9 DNA Cell. Biol. 401, 1990). Subsequent studies indicated that PP14 was present not only in the endometrial decidua and serum of pregnant women, but also in the seminal fluid of men. Applicant has discovered that PP14 is also produced in cells outside of the reproductive tract. PP14 mRNA and protein has been localized not only to a human leukemic cell line induced to differentiate along the megakaryocytic lineage, but also to the end-cell of that lineage, namely, the platelet. Cloning and hybridization analyses of PP14 mRNA, and immunoprecipitation analyses of PP14 protein, have further established that whereas endometrial PP14 is composed of a single dominant species, hematopoietic PP14 (as detected in PMA-induced K562) is comprised of two codominant species. One of the PP14 mRNA species comigrates with endometrial PP14 mRNA, whereas the second one is shorter. The latter contains an internal deletion that is predicted to yield a 22 amino acid deletion in the encoded protein. To simplify considerations of these two mRNA species and their encoded protein products, the undeleted and deleted variants have been here given the designations PP14.1 and PP14.2, respectively.

The identification of a suitable splice acceptor consensus sequence at the site within exon-2 where the deletion of PP14.2 terminates indicates that this variant arises through alternative splicing of the PP14 gene.

The observation that PP14.1 and PP14.2 polypeptides, as well as their corresponding mRNAs, occur in equimolar amounts in PMA-induced K562 cells is consistent with the notion that in the case of cells of the megakaryocytic lineage, the two form heterodimers. Previous work has suggested that endometrial PP14, which consists of PP14.1, consists of weak homodimers which are readily dissociated by antibody. The appearance of both PP14.1 and PP14.2 in immunoprecipitates from cells of the megakaryocytic lineage suggests either that the two form a relatively stable

heterodimer which is not dissociated by antibody, as is the PP14.1:PP14.1 homodimer, or that the monoclonal antibody cross-reacts with the two isoforms. Since there is evidence that PP14.1 may not function as a monomer and the PP14.1 homodimer is easily dissociated, a PP14.1:PP14.2 heterodimer, as disclosed in this invention, provides an alternative to the homodimer which is more stable for use in immunotherapeutic applications. Moreover, the data do not rule out the presence of PP14.2 homodimers which also may have higher stability than PP14.1 homodimers.

K562 (available from the American type culture collection) human myeloid leukemic cells, can be induced to differentiate along the megakaryocytic lineage by phorbol 12-myristate 13-acetate (PMA), and provides a model cellular system for addressing molecular issues in megakaryocytopoiesis. Equivalent cell lines can be isolated by methods known in the art. Megakaryocytic markers known to be expressed by such cells include platelet glycoprotein IIIa (gpIIIa), platelet-derived growth factor (PDGF) alpha and β chains, and transforming growth factor β (TGFβ) (Alitalo, 14 Leukemia Res. 501, 1990). In order to study changes in the mRNA expression profile that accompany K562 differentiation along the megakaryocyte lineage, and to clone and identify novel genes associated with megakaryocyte differentiation and platelets, differential cDNA screening was applied to clone mRNAs that are differentially expressed post-PMA induction of the K562 cell line.

Example 1: Myelogenous Cell Differentiation

The human myelogenous leukemia cell line K562 (ATCC 243) was maintained in RPMI medium (Whittaker Bioproducts) supplemented with 10% heat-inactivated fetal calf serum (FCS), 10mM HEPES, pH 7.2, 40μg/ml gentamycin, and 2mM glutamine. Cells were grown at 37°C with 5% CO_2. The phorbol ester used to cause differentiation was phorbol 12-myristate 13-acetate (PMA, Sigma) which was diluted in dimethyl sulfoxide (DMSO) and stored at -20°C at 1mM-3mM

until use. Final concentrations of PMA ranged between 100nM and 10nM. To determine the effect of PMA on K562 differentiation, K562 cells were cultured at $2x10^5$ cells/ml in tissue culture flasks. PMA was added directly to the flask and cells were harvested 24, 48 or 72h after addition.

Example 2: Cloning PP14 Genes

A phage cDNA library was constructed from a pool of three groups of K562 cells alternatively induced with PMA for 24, 48, and 72 hour. Such a pool was chosen to maximize chances of finding different genes activated throughout the stochastic K562 differentiation program. Duplicate lifts of the "induced" K562 cDNA library were differentially screened with subtracted (induced minus uninduced K562) and nonsubtracted (uninduced K562) single-stranded probes.

Total RNA was isolated from cells using a standard guanidinium isothiocyanate/cesium chloride method. Poly (A)+ RNA was isolated using oligo (dT) cellulose. A cDNA library was constructed using 1.5μg poly (A)+ RNA from K562 cells treated for 24, 48 and 72h (4.5μg poly (A)+ RNA total) with PMA (100nM). The library was constructed using the Uni-ZAP XR cloning kit according to manufacturer's recommendations (Stratagene, La Jolla, CA).

To differentially screen the induced K562 cDNA library, 60,000 individual primary clones were used to infect PLK-F' bacteria (Stratagene). Duplicate membrane lifts were taken using Stratagene Duralon U.V. membranes. The DNA was denatured by incubating the membranes in 1.5M NaCl-0.5M NaOH for 4 min., neutralized for 5 min. in a solution of 1.5M NaCl-0.5M Tris-HCl (pH 8.0), and finally rinsed in 0.2M Tris-Cl (pH 7.2)-2x SSC (1x SSC: 150mM NaCl-15mM sodium citrate, pH 7.0). The DNA was UV cross-linked using the UV Stratalinker 1800 (Stratagene) and filters were allowed to dry.

Membranes for the primary screens were hybridized with non-subtracted single-stranded cDNA probe from untreated K562 cells (uninduced) and compared to duplicate lifts hybridized with subtracted single-stranded cDNA probe from PMA-treated K562 cells (induced). Secondary and tertiary screens were hybridized with single-stranded cDNA probe from untreated K562 cells and compared to duplicate lifts hybridized with non-subtracted single-stranded cDNA probe from PMA-treated K562 cells. To generate the uninduced cDNA probe and the non-subtracted induced cDNA probe, 1μg poly (A)+ RNA from untreated K562 cells or from PMA-treated K562 cells, respectively, was reverse transcribed for 1h at 37°C in a buffer containing 0.1mM oligo $(dT)_{12-18}$ primer, 0.5mM each dATP, dGTP,dTTP, with 100μCi $\{\alpha\text{-}^{32}P\}$dCTP (Amersham, Arlington Heights, IL), 10U Superscript reverse transcriptase (Bethesda Research Labs, Gaithersburg, MD), 50mM Tris-HCl (pH 8.3), 75mM KCl, 3mM $MgCl_2$, and 0.1mM DTT. Unlabeled dCTP was added to 0.5mM for the last 15 min. Following this RT reaction, RNA was hydrolyzed in 100mM NaOH for 30 min. at 65°C and the single-stranded cDNA passed over a Bio-Spin 30 column (Bio-Rad, Richmond, CA) to eliminate free nucleotides.

To generate the subtracted, induced cDNA probe, 0.33μg poly(A)+ RNA from K562 cells treated with PMA for 24, 48, and 72 was pooled (1.0μg of poly (A)+ RNA total) and reverse transcribed as described above, but with 250μCi $\{\alpha\text{-}^{32}P\}$dCTP. Following RNA hydrolysis and cDNA precipitation, the single-stranded cDNA was annealed with a 30-fold excess of photobiotinylated poly (A)+ RNA from untreated K562 cells and subtracted according to manufacturer's suggestions (Invitrogen, San Diego, CA).

Membranes were prehybridized at 42°C overnight in 50% deionized formamide, 1% sodium dodecyl sulfate (SDS), 10% dextran sulfate (MW 300,000), 1M NaCl, and 150μg/ml of denatured sheared salmon sperm DNA (Sigma). Single-stranded cDNA probe was added and incubated for 48h at 42°C in a rotating oven (Hybaid, Middlesex, UK). Lifts

were washed 2x in 2x SSC, 1% SDS at 22°C for 10 min.; then 3x in 0.1x SSC, 0.1% SDS at 65°C for 30 min. Membranes were exposed to Kodak AR film as necessary using intensifying screens at -70°C.

Approximately sixty-thousand cDNA clones were analyzed, and 127 putative positives were selected after the first round of screening. Two of the 127 cDNA clones were identified as PP14, after analysis and sequencing of all the clones in a random fashion and subsequent sequence comparisons with the Genbank sequence database (Pearson et al., 85 Proc. Natl. Acad. Sci. USA 2444, 1988).

Example 3: PP14 mRNA Expression

Verification of PP14 mRNA expression in the K562 line, and confirmation of its PMA-inducibility, was accomplished by RNA transfer blot analyses (see figures 1 and 2).

Total RNA (10μg) was isolated as described above, heated to 65°C for 15 min. in 50% formamide, 6% formaldehyde, 1x EPPS (N-(2-hydroxyethyl)- piperazine-N'-3-propanesulfonic acid) buffer (1x EPPS buffer: 20mM EPPS (pH 8.2), 10mM Na-acetate (pH 5.2), 2 mM EDTA) and separated on 1.2% agarose gels containing 1x EPPS buffer and 6% formaldehyde. The RNA was passively transferred to Duralon UV membranes (Stratagene) and UV crosslinked. Membranes were prehybridized as described above. Probe was generated by random priming 20ng of purified PP14 DNA. The DNA was denatured by heating to 100°C for 10 min., then incubated for 30 min. at 37°C in 50μCi {^{32}P}dCTP (Amersham), 0.2mM each dGTP, dTTP, dATP and 10U Klenow enzyme in a buffer containing random hexanucleotides (Boehringer Mannheim, Indianapolis, IN). Probe was hybridized overnight at 42°C. Membranes were washed and exposed as described above.

Specifically, cellular RNA from uninduced K562 cells or from K562 cells induced with PMA (10nM) for 0.5, 1, 2, 3, 6, 12, and 24 hours was hybridized with a labeled PP14

cDNA probe. PP14 mRNA was undetectable in uninduced K562 cells and became apparent within 6 hours of PMA treatment (Fig. 1). PP14 mRNA reached plateau levels at 24h and remained relatively constant out to at least 72h (Figs. 1 and 2). A single broad PP14 mRNA band corresponding to a length of approximately 0.8 kilobases was observed in all cases, coinciding with the size reported for endometrial PP14 mRNA.

Example 4: Cell Specificity of PP14

In order to determine whether the PP14 induction event is cell-, lineage-, and/or inducer-specific, several leukemic lines were induced with alternative chemical inducers (Fig. 2). Whereas PMA-induction of K562 along the megakaryocytic lineage was associated with high levels of PP14 mRNA accumulation, hemin (50uM)-induction of K562 cells along the erythroid lineage (Andersson et al., 278 Nature 364, 1979) demonstrated no similar PP14 activation. HL-60, another bipotential leukemic line, was tested for PP14 inducibility. Neither PMA-induced HL-60 cells, which differentiate along the myelo-monocytic lineage (Rovera et al., 76 Proc. Natl. Acad. Sci. USA 2779, 1979) or DMSO-treated HL-60 cells, which differentiate along the neutrophilic lineage, displayed detectable PP14 expression. Similarly, PMA induction of two other leukemic lines, U937 and PLB-985, along the myelo-monocytic lineage failed to elicit PP14 mRNA expression. The intactness of the mRNA used from these various leukemic lines was confirmed using an actin probe (data not shown). Hence, PP14 does not seem to simply be a phorbol ester-responsive gene. Moreover, these data suggest that PP14 is not promiscuously expressed in leukemic cells. Instead, these data are consistent with the notion that there is a specific association between PP14 and the megakaryocytic lineage of PMA-induced K562 cells.

Example 5: PP14 Protein Expression

To determine whether hematopoietic PP14 mRNA is in fact translated into PP14 protein, immunoprecipitation analyses were performed.

K562 cells were cultured at $5x10^6$ cells/ml and treated with PMA as described above. Cells were labeled with 0.5mCi ^{35}S-cysteine and ^{35}S-methionine (ICN Biomedicals, Inc., Irvine, CA) in cysteine- and methionine-free media supplemented with 10% FCS which had been extensively dialyzed in PBS. The next day, cells were collected by centrifugation and conditioned media were saved. Cells were lysed in 1% triton X-100, 1% bovine serum albumin (BSA) and 1mM phenylmethylsulfonylfluoride (Sigma). Conditioned media were used directly. After pre-clearing cell lysates or conditioned media with protein-G Sepharose (Pharmacia, Piskataway, NJ), 100μl of a 50% slurry of protein-G Sepharose was added with 2μl of mouse monoclonal anti-PP14 antibody (105DH1F1; Riittinen et al., 136 J. Immunol. Meth. 85, 1991). After incubating overnight at 4°C with gentle rotation the beads were pelleted by centrifugation and washed as follows: 5x in 0.1% triton X-100, 0.1% BSA; then 1x in 0.01M Tris-HCl (pH 8.0), 0.14M NaCl, 0.025% NaN_3; and finally 1x in 0.01M Tris-HCl (pH 7.5). Bound protein was eluted by boiling and electrophoresed on a 3% stacking, 15% resolving SDS-PAGE gel. The gel was dried and exposed to Kodak XR film at -70°C with an intensifying screen.

K562 cells were metabolically labeled with a combination of ^{35}S-cysteine and ^{35}S-methionine. Conditioned medium and cell lysis extracts were then immunoprecipitated using a mouse monoclonal anti-PP14 antibody, or a polyclonal antibody. Only conditioned medium from PMA-induced, but not uninduced, K562 cells demonstrated PP14 protein (Fig. 3). Significantly, two protein species were noted in the 28 kilodalton size range, with the upper one comigrating with purified endometrial PP14 (data not shown). The two PP14 isoforms were present in approxi-

mately equivalent amounts. No PP14 was seen in cell lysis extracts of uninduced K562 cells and only small amounts of PP14 were detected in the extracts of induced K562 cells, consistent with PP14's being a secreted protein (data not shown). Hence, PP14 protein parallels PP14 mRNA in its PMA-inducibility in K562 cells.

Example 6: Biological Effect of PP14

In order to assess the effect of conditioned medium from K562 cells on peripheral blood lymphocyte proliferation, two-way mixed lymphocyte cultures (MLC) were set up as follows. The blood from two unrelated donors was collected in heparin (10U/ml blood). Blood was diluted two times in 1x PBS; 0.3 vol. ficoll-paque (Pharmacia, Uppsala, Sweden) was under-layered and then centrifuged for 30 min. (1000 x g). The leukocyte layer was removed, washed three times with 1x PBS, and lymphocytes counted in a hemocytometer. Cells were cultured in 96 well plates at 2 $x10^5$ cells/well in triplicate. Supernatants, where used, were added to 50% v/v in cell culture wells. Antibody was added directly at a 1:100 dilution. Cells were cultured for 5-7 days as described above. Twelve to fifteen hours before harvesting 0.5μCi [^{3}H]thymidine (New England Nuclear, Boston, MA) was added to each well. Cells were harvested onto filters with a MiniMash II cell harvester, the filters were dried, and counted in 1ml scintillation cocktail with a Beckman (Fullerton, CA) LS3801 beta counter.

In previous studies, endometrial PP14 has been shown to be a potent suppressor of lymphocyte proliferation. In order to determine whether the PP14 originating from hematopoietic cells shared this immunoregulatory function, conditioned medium from PMA-induced K562 cells was added to two-way mixed lymphocyte cultures, and the effect on proliferation, as measured by ^{3}H-thymidine incorporation on days 5, 6, and 7, was monitored. In these experiments, conditioned medium was added to the MLC at a 1:2 ratio.

In positive control MLCs, when lymphocytes from two unrelated individuals were cocultured, a high degree of proliferation, peaking on days 6 and 7, was detected (data not shown). Addition of conditioned medium from 24h (Fig. 4) or 48h (data not shown) PMA-induced K562, but not from uninduced K562, resulted in significant inhibition of proliferation.

Example 7: Antibody blocking of PP14

In order to verify that the lymphocyte proliferative inhibition effected by PMA-induced K562 conditioned medium was indeed due to the presence of PP14 protein, antibody-blocking experiments were performed. A rabbit polyclonal antiserum against purified PP14 and which is known to effectively immunoprecipitate PP14 protein was added to the cocultures containing K562 conditioned medium. Anti-PP14 antiserum (1:100 dilution) completely reversed the anti-proliferative effect of the conditioned medium by day 7 for both 24h- (Fig.4) and 48h-induced (data not shown) K562 conditioned medium. Hence, PP14 is clearly implicated in the K562 immunosuppressive effect.

The anti-proliferative activity of PMA-induced K562 conditioned medium was unaffected by normal rabbit serum or a neutralizing anti-transforming growth factor (TGF) β monoclonal antibody (R&D Systems, Minneapolis, MN) (data not shown). Since TGFβ has been reported to be produced by K562 cells, and furthermore, since TGFβ is known to inhibit lymphocyte proliferation, one can presume that the TGFβ that is present has not been cleaved into its active immunomodulatory form. Significantly, PP14 does not require proteolytic cleavage for functional activation.

Example 8: mRNA species of PP14 isoforms

While the immunoprecipitation analyses clearly distinguished between two PP14 polypeptide isoforms, RNA transfer blot analyses did not clearly resolve distinct PP14 mRNA species corresponding to the polypeptide iso-

forms. Reexamination of weaker exposures of the RNA transfer blots, however, suggested the presence of a tight doublet hybridizing to the PP14 probe. In order to investigate this in more detail, reverse transcriptase polymerase chain reaction (RT-PCR) cloning and analyses were performed.

For PCR analyses of PP14 mRNA, total RNA was reverse transcribed as described above using a PP14 specific primer (5'-GGATCCCATGCTCCAAGGGTTTATTAATAACCTCTGC-3'; Seq. I.D. No. 1). Resulting product was PCR amplified with a 5' primer (5'-GGTACCGCTCCAGAGCTCAGAGCCACCCACAG-3'; Seq. I.D. No. 2) and a 3' (5'-GTGCAGAACGATCTCCAGGTTG-3'; Seq. I.D. No. 3) primer in a buffer containing 25mM TAPS-HCl (pH 9.3), 50mM KCl, 2mM $MgCl_2$, 1mM DTT, 200μM each dATP, dCTP, dGTP, dTTP, and 2.5U Taq polymerase (Perkin Elmer, Norwalk, CT). Amplification was performed on a PTC-100 thermal cycler (MJ Research, Watertown, MA) for 25 cycles of 1 min. at 94°C, 45 sec. at 65°C, and 1 min. at 72°C. PCR products were analyzed on a 1.2% agarose gel stained with EtBr.

The initial two PP14 cDNA clones obtained through differential cDNA screening (vide supra) proved to be only partial clones encompassing the 3'-end of PP14 mRNA only. PCR cloning of full-length PP14 cDNA was performed by annealing a PP14 3'-end specific primer (5'- CATGCTCCAAGGG TTTATTAATAACCTCTGC-3'; Seq. I.D. No. 4) and reverse transcribing as described above. The resulting product was used directly in a PCR (described above) using the 3'-end specific primer and a 5' -end specific primer (5'-AGCTCAGA GCCACCCACAGCCGCAG-3'; Seq. I.D. No 5). The PCR product was gel purified and cloned into a T-vector.

A full-length PP14 cDNA was RT-PCR cloned from PMA-induced K562 mRNA using primers based upon the 3'-end sequence of K562 PP14 mRNA (identical to the endometrial PP14 mRNA sequence) and the published 5'-end sequence of endometrial PP14 mRNA. DNA sequencing was performed using the Tabor and Richardson sequencing method, and the

SEQUENASE® sequencing kit as per the manufacture's recommendations (United States Biochemicals, Cleveland). Sequence analysis of the K562 PP14 clone revealed an encoded PP14 protein identical to endometrial PP14, with the significant difference of a 66 nucleotide long in-frame deletion resulting in a predicted 22 amino acid deletion in the encoded protein (Fig. 5). When the genomic PP14 structure was analyzed, this deletion corresponded to the upstream end of exon-2, and a consensus splice acceptor site was detected at the appropriate site within exon-2. This points to alternative splicing as the operative mechanism in the generation of the shorter PP14 mRNA variant.

When the PCR product using PP14 end primers was size-fractionated and visualized on agarose gels, two distinct PCR products were noted (Fig. 6). The two mRNA species were designated PP14.1 and PP14.2, with the latter corresponding to the smaller RNA species. The size difference between these two PCR products was consistent with the 66 nucleotide deletion documented in the K562 PP14 mRNA that had been PCR-cloned. Notably, only the PP14.1 mRNA variant could be readily detected in endometrial tissue.

Example 9: Detection of PP14 Proteins in Platelets

Immunoprecipitation from platelets was performed from platelets isolated using a modified citrate buffer. Briefly, whole blood was collected in a citrate/phosphate/dextrose/adenine anticoagulant 1.4ml CPDA/10ml whole blood (CPDA contains 15.8 mM citric acid, 91.3mM sodium citrate, 16.6mM dextrose, 1.7mM adenine, and 16.6mM monobasic sodium phosphate). Cells were spun at 200 x g, for 10 min. at 22°C. The upper platelet rich plasma (PRP) was removed and the platelets were washed two times in regular ringers/citrate/dextrose, pH 6.5 (RCD, containing 71.9mM NaCl, 0.7mM KCl, 0.6mM $CaCl_2$, 0.8mM $NaHCO_3$, 20mM trisodium citrate, 27.8mM dextrose, 43ng/ml prostaglandin E_1 (PGE_1 Sigma Chemical Co, St. Louis)). Between washes, platelets

were spun at 1,100 x g for 15 min. at 22°C. Reductive methylation of platelet extracts was performed by resuspending the platelet pellet after the final spin in a modified lysis buffer containing 50mM sodium phosphate (pH 7.0), 1% NP-40,150mM NaCl, 2μg/ml leupeptin, 2μg/ml aprotinin, 20μg/ml PMSF. $NaCNBrH_3$ (Sigma) was added to 50mM and [^{14}C]formaldehyde (Amersham) was added to 10mM. The mixture was allowed to reductively methylate for 1h at 37°C. Following methylation the reaction was dialyzed overnight in 1x PBS, then again the next morning for 1h in 1x PBS to remove remaining $NaCNBrH_3$ and [^{14}C]formaldehyde. After dialysis, the radioactive extracts were immunoprecipitated and analyzed as described above.

As stated above, the finding of PP14 in PMA-induced, but not hemin-induced K562 leukemic cells, along with the absence of PP14 in other PMA-induced leukemic lines, together substantiated that PP14 expression in PMA-induced K562 cells reflects an underlying association between PP14 and the platelet lineage. To confirm this point, immunoprecipitation/SDS-PAGE analysis was performed using polyclonal anti-PP14 antibody and platelet extracts. Evidence for the presence of the two PP14 polypeptides in the platelet extract was obtained (data not shown).

Example 10: Screening for PP14 by PCR

The finding of PP14 mRNA and protein in hematopoietic cells of the megakaryocytic lineage prompted screening of additional tissue sites outside of the reproductive tract for the presence of PP14 mRNA. PCR analysis was used to optimize sensitivity. Total cellular RNA, isolated from autopsy tissues, was reverse-transcribed using PP14-specific primer (Seq. I.D. No. 1) and amplified using the same oligonucleotide as 3′ primer, and a 5′ primer (Seq. I.D. No. 2) designed to allow resolution of the two PP14 mRNA variants. As seen in Fig. 7, PP14.1 and PP14.2 were detected, and clearly resolved, in the PMA-induced K562 cells serving as controls (lanes 2 and 3). Moreover, the

placental tissue (contaminated with PP14-containing contaminant endometrial decidua) showed, as expected, only the larger PP14.1 mRNA variant (lanes 11 and 12). In contrast, the panel of other tissues examined were all negative for PP14 transcript. These tissues included brain (lane 4), spleen (lane 5), small intestine (lane 6), liver (lane 7), kidney (lane 8), and meninges (lane 9). Similarly, two fibroblastic cell lines, KM-102 (human bone marrow stromal cells; lane 10) and dermal fibroblasts (lane 11) were also negative. Hence, PP14 mRNA expression seems to be restricted to the reproductive and hematopoietic systems.

Methods

The presence of a constitutively active immunosuppressive molecule in cells of the platelet lineage is of considerable physiological significance, and indicates previously unsuspected roles for PP14. These data indicate that this potent immunosuppressive molecule is concentrated in tissue sites where coagulation occurs. Thus, PP14 may play a role in the resolution of inflammatory processes at wound healing sites. This discovery thus shows a previously unknown critical molecular link between the coagulation and immune systems.

The discovery that PP14 is produced by cells of the platelet lineage leads to novel therapies for reversing platelet-driven immunosuppression. PP14 was previously thought to be a beneficial molecule, in its suggested physiological role as a blocker of alloresponses in the female and male reproductive tracts. The present invention discloses that PP14 can also be a deleterious molecule, in its pathophysiological role as a potent general immunosuppressive agent released by platelets in certain clinical settings, such as coagulopathies. This discovery provides the first motivation for developing methods for blocking PP14 and its immunosuppressive effects. Methods are disclosed for carrying out this PP14 blockade. These

methods involve both methods for interfering with PP14 protein interaction with a PP14 receptor, and methods for preventing PP14 transcription and/or translation (i.e., PP14 blockade therapies).

The survey of human leukemic cell lines reported in this study indicates that PP14 is not promiscuously expressed in leukemic lines. In fact, even in K562, it is only expressed following chemical induction with one particular chemical inducer. Nonetheless, it is possible that under in vivo conditions, myeloid leukemic cells that share K562's megakaryocytic differentiative potential, may be triggered into a more differentiated state wherein they can express PP14. This could occur spontaneously or in response to therapeutic agents. Under such conditions, PP14, by virtue of its potent immunosuppressive function, is expected to play a pathogenic role in blocking effective anti-tumor immune responses. As was shown here for K562 leukemic cells, the secreted PP14 is in its biologically active form. The present invention provides methods for detecting PP14.2 in serum of patients. Such methods can readily be applied to patients with leukemia or related diseases in order to determine which patients are candidates for PP14 blockade therapy.

There follow examples of the therapeutic and diagnostic methods of the invention below. These examples are not limiting in the invention and those of ordinary skill in the art will recognize that many equivalent methods and reagents can be discovered within the scope of the claims.

PP14 Blockade Therapy

Patients with elevated levels of one or both of the PP14 polypeptide isoforms in their serum are candidates for PP14 blockade therapy. Such elevated levels of PP14 may occur in a variety of disease conditions where platelets release excess PP14 into the bloodstream. For example, sepsis is often associated with disseminated intravascular coagulation,. a condition which leads to the

release of platelet contents into blood as the platelets coagulate. PP14 that is released in this clinical setting contributes to generalized immunosuppression which, in turn, further aggravates the sepsis. Hence, PP14 blockade serves to interfere with this pathogenic cycle and aid recovery.

The first step is to identify a patient in need of PP14 blockade therapy. Preferred diagnostic methods for accomplishing this are described below. Generally, those individuals having higher than normal tissue or serum levels of PP14.1 or PP14.2 or both are treated by methods of this invention. Such levels can be determined by use of antibody-based assays.

The present invention discloses that PP14 blockade can have therapeutic benefit. Methods for blocking PP14 action include standard approaches for blocking proteins to achieve therapeutic endpoints. For example, monoclonal and/or polyclonal antibodies can be used with specificity for the two isoforms of PP14 as PP14 blocking agents. Antibodies with specificity for PP14 have previously been described, but, the only suggested therapeutic benefit was for treatment of an immune system disorder, the only given example of which was AIDS, and in the absence of knowledge about the two PP14 isoforms, the isoform-specificity of the antibodies remained unknown. Knowledge of the PP14.2 isoform in the present invention permits the development of antibodies with PP14.1 or PP14.2-specificity and their therapeutic use. Thus, this invention features methods to treat all other non-AIDS- diseases (or even other non-immune system disorders) characterized by excess amounts of PP14 in a patient or tissue. It also features use of PP14.2-specific antibodies for treatment of all diseases associated with elevated PP14 levels.

A preferred strategy for producing anti-PP14.1-specific antibodies is to immunize with a peptide largely limited to the amino acid sequence within the PP14.1-specific sequence that is absent from PP14.2. Alternatively, pep-

tides overlapping the junctions between this sequence and the rest of PP14.1 can be used, as well as other PP14.1 sequences. In the latter case, antibodies with cross-reactivity to both PP14.1 and PP14.2 can be eliminated by conventional approaches.

A preferred strategy for producing anti-PP14.2-specific antibodies is to immunize with a peptide spanning the junction corresponding to the site where the PP14.1 sequence insertion is present. Standard peptide immunization protocols can be employed.

PP14 peptides having only a portion of the native PP14 polypeptides can be used to competitively inhibit interaction of PP14 with a receptor for PP14 (a PP14 receptor) to thereby interfere with the nonspecific immunosuppression mediated by the native PP14 polypeptide. Straightforward in vitro experiments based upon competitive peptide inhibition and/or site-specific mutagenesis can be used to localize the appropriate PP14 subsequences for effecting such blockade, and can be carried out based upon standard protocols by those familiar with the art. Preferred subsequences are generally those coinciding with hydrophilic amino acid sequence stretches. Moreover, strategies for optimizing in vivo dosing schedules for patients to be treated are well-known.

Similarly, anti-idiotypic antibodies that mimic PP14 in its capacity to bind to its receptor can be used for competitive blockade of a PP14 receptor. Methods for preparing such anti-idiotypic antibodies and their use are well-known to those familiar with the art, and are particularly well-documented in the infectious disease literature.

The PP14 receptor, instead of PP14 itself, can also be focussed upon for purposes of blocking the PP14:PP14 receptor interaction. The purification and cloning of receptors, in situations where the ligand is available in purified form, has become a straightforward exercise for those familiar with the art. Prior to this invention, the

art provided no compelling therapeutic reason to purify and clone the PP14 receptor. Moreover, in the absence of prior knowledge of the existence of two distinct PP14 isoforms, the issue of isoform-specificity for PP14 receptors could not be addressed. The present invention, by teaching that PP14 is not simply a byproduct of pregnancy or restricted to a distal site of the male reproductive tract, but instead can be derived from platelets which contribute to pathophysiology, provides a compelling reason to purify and clone receptors for PP14. Structural characterization of the PP14 receptor allows, in turn, the generation of agents for blocking PP14:PP14 receptor interaction.

A preferred method for isolation and cloning the PP14 receptor involves generation and use of a PP14:immunoglobulin Fc chimeric polypeptide. This chimeric polypeptide has the complete sequence of PP14, or a functional polypeptide derivative thereof, linked to the Fc region of immunoglobulin G1 (IgG1). The latter serves as a useful tag for detecting and isolating the chimeric polypeptide, since the Fc region of immunoglobulin binds well to protein A or protein G. Analogous ligand:Fc chimeric polypeptides have been used by a number of investigative groups to isolate specific receptors. Hematopoietic cells, such as monocytes and lymphocytes, respond to PP14 and hence the mononuclear cell pool is a suitable cellular source from which to isolate PP14 receptors. A detergent extract is prepared from said cells, and the PP14:immunoglobulin Fc chimera, produced by recombinant DNA methods in standard NOS cells or their equivalent, is added to the extract. Protein A-sepharose chromatography is performed to isolate a complex in which the PP14 receptor complexed to the PP14:immunoglobulin Fc chimera. pH elution is used to recover the purified PP14 receptor. Amino acid sequence for the amino-terminus, as well as for defined sub-peptides generated by peptidase cleavage, is determined by conventional amino acid sequencing methods.

Based upon this amino acid sequence, degenerate oligonucleotides encoding this amino acid sequence are synthesized using an oligonucleotide synthesizer. These degenerate oligonucleotides are then labeled and used as probes to screen a cDNA library from the same cells from which the receptor was originally purified. Hence, purification and cloning of PP14 receptors can be carried out in a straightforward way using conventional methods.

While the example of using PP14:Fc chimeras as a receptor trap is provided here, there are multiple alternative approaches that can be employed to the same end. Alternative polypeptide tags can be appended to PP14 for detection and isolation purposes. In addition, the sequence of steps outlined here can be repeated for both PP14.1 and PP14.2, as well as heterodimeric PP14, polypeptides in order to isolate and clone isoform-specific receptors. Moreover, native PP14 can be used in unmodified form for binding to the receptor, and anti-PP14 antibodies can then be used to recover the PP14:PP14 receptor complex.

According to one therapeutic method for effecting PP14 blockade via the PP14 receptor, anti-PP14 receptor antibodies are infused into a patient in need of PP14-induced generalized or localized immunosuppression. Anti-PP14 receptor antibodies can be generated using PP14 receptor, recombinant PP14 receptor or PP14 receptor peptides as immunogens. Methods for preparing monoclonal antibodies useful for human therapeutics are well-described in the scientific literature.

According to another therapeutic method for effecting PP14 blockade via the PP14 receptor, a soluble derivative of the PP14 receptor is infused into a patient in need of PP14-induced generalized immunosuppression. This soluble derivative of the PP14 receptor can be readily generated through standard recombinant DNA methods. For example, a site-specific mutagenesis method can be employed to introduce a stop codon at the carboxy-terminus of the PP14

receptor extracellular domain. This mutagenized coding sequence for a soluble PP14 receptor can be subcloned into any one of a number of available eukaryotic or prokaryotic expression vectors for quantitative production of this molecule. An alternative therapeutic soluble derivative of PP14 receptor comprises the extracellular domain of the PP14 receptor linked to the Fc domain of immunoglobulin G. This latter molecule has the advantage of being a more stable molecule in vivo, as has been shown for other immunoglobulin Fc chimeric polypeptides, such as CD4: immunoglobulin Fc.

Therapy Based Upon Inhibition of PP14 Production

In addition to therapeutic methods based upon binding of the PP14 protein in the blood and tissues of a patient, and thereby interfering with its immunosuppressive activity, therapeutic methods can also be used which are based upon reducing levels of PP14 production. Such a reduction can be at transcriptional and post-transcriptional levels.

Thus, genetic therapeutic agents that interfere with PP14 expression by cells of the platelet lineage can be used. A preferred agent for reducing PP14 production is an antisense PP14 oligonucleotide that has been covalently derivatized through known methods to enhance in vivo stability and cell membrane penetration. Isoform-specific antisense PP14 oligonucleotides can readily be designed based upon the PP14.1 and PP14.2 nucleotide sequence information disclosed herein, along with general guidelines that are well-known to those familiar with the art for optimizing functional antisense oligonucleotides. In the case of PP14.1, the antisense oligonucleotide can be directed against the 66 nucleotide stretch that is absent in PP14.2. In the case of PP14.2, the antisense oligonucleotide can span the junctional site that overlaps the 66 nucleotide deletion of PP14.1 sequence such that the oligonucleotide binds only to this junction. Other regulatory polynucleotides can be readily designed, such as

ones that incorporate ribozymes or that function as triplex-forming regulatory elements. There are well-established procedures for developing such agents and for screening their efficacies and toxicities.

Other therapeutic methods can be developed that are based upon chemical agents that function as PP14 gene-specific transcriptional inhibitors. There are currently well-described methods for screening banks of chemicals for ones that will block transcription from a specific gene promoter. One experimental approach involves generating a reporter gene construct including the upstream sequence of the PP14 gene, with the PP14 promoter element, linked to a gene sequence encoding a readily detectable promoter, such as luciferase, beta-galactosidase, or chloramphenicol acetyltransferase. This transcriptional cassette is stably transfected into a target cell that is capable of supporting active transcription from the PP14 promoter. K562 cells are one example of a suitable cell line for this purpose. Alternative cell lines with megakaryocytic differentiation potential, as well as endometrial and testicular lines, can be readily identified. In the case of K562 cells transfected with the chimeric reporter, the cells are distributed into multi-well tissue culture plates, and chemicals to be screened are added to individual wells, along with PMA to activate the PP14 promoter. Wells containing cells failing to express the reporter are identified. This represents a relatively rapid method for screening large numbers of chemicals for relevant drug candidates. Once a candidate is identified, procedures well-established in the field of pharmacology are used to study efficacy and toxicity and to optimize dosage regimens.

Treatment of Leukemia

PP14 characterizes cells of the platelet lineage, and leukemic cells corresponding to this lineage also express PP14. Leukemic cells producing this potent immunosuppres-

sive molecule in vivo may be protected from effective anti-leukemic T-cell responses. Moreover, PP14 derived from these cells will lead to generalized immunosuppression in the patient, rendering the patient susceptible to other diseases, including infectious diseases. Hence, the discovery that certain leukemic cells produce PP14 provides motivation for screening patients with leukemia for PP14 expression in blood and leukemic cells. Those patients who are PP14-positive, can be treated by PP14 blockade therapy, by one of the methods discussed above. Patients with megakaryocytic leukemia or chronic myeloid leukemia cells with megakaryocytic differentiation potential, are particularly suitable candidates for this mode of therapy.

The present invention discloses the existence of the PP14.2 isoform. This disclosure is significant in that it teaches those skilled in the art how to effectively block platelet-derived PP14, in that both isoforms must be targeted with antibody, PP14 peptide, or PP14 receptor derivatized therapeutic compositions. Cloning of the PP14.2 isoforms has provided critical sequence information which allows the generation of PP14 isoform-specific reagents. Such reagents can be used either in combination or independently. For example, therapeutic benefit may be obtained in a pregnant patient who is in need of amelioration of platelet-induced generalized immunosuppression, as for example in the clinical context of a pregnancy-associated coagulopathy. Since in such a patient, PP14.1 blockade is undesirable due to potential adverse effects upon the developing fetus, selective PP14.2 blockade is preferable. A therapeutic benefit will be derived by decreasing the PP14 load in the patient, even if there is residual PP14.1 from endometrium and platelets in the patient.

PP14 Diagnostic Assays

The disclosure of PP14 in cells of the platelet lineage has diagnostic implications. Monoclonal and/or poly-

clonal antibodies, prepared by the methods described above, permit the simple development of ELISA assays for measuring PP14 expression in serum. PP14.1- and/or PP14.2-specific antibodies can be used for this purpose. A sensitive ELISA can be used for PP14 detection in serum samples. Two anti-PP14 antibodies with specificities for distinct, non-overlapping portions of PP14 protein, can be combined in a conventional double-antibody sandwich ELISA. The present invention teaches that two isoforms of PP14 are produced by platelets, and hence, informs those skilled in the art how to develop suitable diagnostic assays. The ability to distinguish between the PP14.1 and PP14.2 polypeptide isoforms is of particular utility when diagnosing platelet disorders in pregnant women, since the PP14 polypeptides deriving from platelet and endometrial cells can be resolved.

Not only is this assay useful for precisely defining candidates for PP14 blockade therapy, but in addition, this assay can serve as a sensitive diagnostic tool for determining whether there is in fact a coagulopathy in a patient where the diagnosis is uncertain. According to the latter, PP14 detection can serve as a diagnostic test for clinical disorders such as disseminated intravascular coagulation which are often difficult to diagnose. Thus, PP14.2 polypeptide is a useful diagnostic marker for platelets.

Immunosuppression Therapy with PP14.2 Polypeptide

This invention provides a novel immunosuppressive agent that can be used for the treatment of patients with diseases where immunosuppression is the desired endpoint. Such diseases include, but are not limited to, autoimmune diseases, rheumatoid arthritis, allergic disorders such as allergic dermatitis, transplant rejection, and graft-versus-host disease in the context of bone marrow transplantation. The present invention provides PP14.2 as an

alternative to PP14.1 for achieving immunosuppression in patients in need of this.

A patient is identified who is in need of immunosuppression. A composition including a PP14 polypeptide, or a functional polypeptide derivative thereof, is administered to the patient. The route of administration and the precise components of the pharmaceutical preparation are dictated by the particular disease entity being treated. For example, in the treatment of a systemic autoimmune disease, intravascular or intramuscular administration is preferable. In contrast, topical application is preferable for allergic dermatitis, whereas intra-articular injection may be necessary in the context of rheumatoid arthritis. Methods for optimizing clinical protocols involving therapeutic polypeptides are well-established, and the methods to be used for PP14 polypeptides parallel these. For example, an amount of PP14 between 1 and 1000 μ for kg animal per day is suitable. PP14.1 and/or PP14.2 serum levels in treated patients can be monitored in order to provide one measure of therapeutic efficacy.

Methods for PP14 Polypeptide Production

The present invention discloses a significant set of new potential sources for both small and large scale production of not only the novel PP14.2 isoform, but also the PP14.1 isoform. Previously there have been no natural cellular sources from which human PP14 polypeptide might be readily derived. Clearly, the availability of first and second trimester endometrium from cases of abortion is limited by several factors. According to the present invention, platelets can be used as a source for native PP14. The finding of PP14 polypeptide in PMA-induced K562 cells, which proliferate rapidly in cell culture, provides another potential source for native (i.e., non-recombinant) PP14. Significantly, both PP14 isoforms can be obtained from these sources, and these further serve as a source for functional dimeric molecules. Moreover, other

cell lines corresponding to the megakaryocytic lineage are alternative sources for native PP14.

Alternatively, expression constructs for PP14.2 can be introduced into K562 or other megakaryocytic lines for purposes of PP14 overexpression. Recombinant monomeric PP14.2, homodimeric PP14.2, and heterodimeric PP14.1: PP14.2 can all be produced in this way. Such cells provide optimal cellular backgrounds in which proper post-transcriptional processing of PP14 mRNA and protein can take place. Methods for gene transfer and the expression and purification of recombinant proteins are well-established in the art.

Alternative methods can be devised for producing soluble PP14.1 and PP14.2 polypeptides. One preferred method involves the expression of chimeric PP14.1:GPI and PP14.2:GPI polypeptides, including monomeric and heterodimeric forms of these polypeptides, on the surfaces of adherent cells and the recovery of soluble PP14.1 or PP14.2 polypeptides by cleavage of the GPI membrane anchor. This type of method has been used successfully for the production of other soluble molecules and offers the particular advantage of being compatible with continuous-flow cell culture systems.

Other embodiments are within the following claims.

(1) GENERAL INFORMATION:

(i) APPLICANT: MARK L. TYKOCINSKI

(ii) TITLE OF INVENTION: PP14-BASED THERAPY

(iii) NUMBER OF SEQUENCES: 5

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Lyon & Lyon

(B) STREET: 611 West Sixth Street

(C) CITY: Los Angeles

(D) STATE: California

(E) COUNTRY: U.S.A.

(F) ZIP: 02111-2658

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.5" Diskette, 1.44 Mb storage

(B) COMPUTER: IBM Compatible

(C) OPERATING SYSTEM: IBM P.C. DOS (Version 5.0)

(D) SOFTWARE: WordPerfect (Version 5.0)

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

Prior applications total, including application described below:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Warburg, Richard J.

(B) REGISTRATION NUMBER: 32,327

(C) REFERENCE/DOCKET NUMBER: 199/187

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (213) 489-1600

(B) TELEFAX: (213) 955-0440

(C) TELEX: 67-3510

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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(3) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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(4) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

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(5) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
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(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

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(6) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

AGCTCAGAGC CACCCACAGC CGCAG 25

Claims

1. Method for treating a patient suffering from non-AIDS immunosuppression, comprising the step of administering to said patient a reagent that specifically prevents or reduces binding of an isoform of PP14 with a receptor for a PP14 isoform under physiologic conditions.

2. The method of claim 1 wherein said reagent comprises a compound selected from the group consisting of a polyclonal or monoclonal antibody specific for PP14.1; a polyclonal or monoclonal antibody specific for PP14.2, a polyclonal or monoclonal antibody specific for PP14.1 and PP14.2, a receptor for a PP14 isoform, a portion of a receptor for a PP14 isoform; a peptide portion of a PP14 isoform; an anti-idiotypic antibody mimic of PP14 or PP14-binding fragment thereof; and a polyclonal or monoclonal antibody to a PP14 receptor or a receptor binding fragment thereof.

3. The method of claim 2 wherein said peptide portion of a PP14 isoform comprises a portion of the polypeptide defined by amino acids 33 through 54 of PP14.1.

4. The method of claim 2 wherein said reagent comprises a soluble polypeptide derivative of a receptor for PP14 comprising the extracellular domain of a PP14 receptor free of said receptor's native transmembrane and cytoplasmic domains.

5. The method of claim 4 wherein said soluble polypeptide derivative is a PP14 receptor:immunoglobulin Fc chimeric polypeptide.

6. The method of claim 1 wherein said patient suffers from a platelet disorder.

7. The method claim 6 wherein said platelet disorder is one of disseminated intravascular coagulation, platelet-induced immunosuppression secondary to platelet transfusion, and thrombocytosis.

8. The method of claim 1 wherein said patient suffers from leukemia.

9. The method of claim 1 wherein said patient is suffering from an autoimmune disease, rheumatoid arthritis, an allergic disorder, transplant rejection, or graft-versus-host disease.

10. Method for cloning a PP14 receptor, comprising the steps of determining an amino acid sequence of said receptor and screening a library for a clone of said receptor using an oligonucleotide probe corresponding to said amino acid sequence.

11. The method of claim 10, wherein said method comprises a method for cloning a complementary DNA corresponding to a PP14 receptor for PP14 comprising the steps of:

(a) providing a chimeric polypeptide comprising a PP14 polypeptide linked to a polypeptide tag;

(b) contacting said chimeric polypeptide with a cellular extract from a cell expressing a PP14 receptor under conditions suitable for forming a complex comprising said chimeric polypeptide bound to said PP14 receptor;

(c) precipitating said complex by contacting a reagent able to react with said polypeptide tag conjugated to an insoluble matrix with said complex;

(d) recovering said PP14 receptor from said matrix;

(e) determining the amino acid sequence of a portion of said PP14 receptor; and

(f) screening a cDNA library for a PP14 receptor cDNA using an oligonucleotide probe corresponding to the amino acid sequence of said PP14 receptor.

12. Method for producing an antibody with specificity for a receptor for a PP14 polypeptide, comprising the step of immunizing a host with a portion of a receptor for a PP14 polypeptide.

13. Method for blocking immunosuppression in a patient, comprising the step of administering to said patient a reagent that blocks transcription of a PP14 gene and/or translation of a PP14 transcript.

14. The method of claim 13 wherein an antisense oligonucleotide, ribozyme, or a triplex-forming nucleic acid is used to block transcription and/or translation of a PP14 polypeptide.

15. Method for identifying a reagent that blocks transcription of PP14, comprising the step of screening a reagent for its capacity to block a PP14 promoter-driven transcription of a reporter gene.

16. A diagnostic method for identifying a patient with a platelet disorder, comprising the step of contacting a sample from said patient with an antibody with specificity for PP14.1 and/or PP14.2, and determining the amount of reaction of said antibody with said sample compared to the amount of reaction observed in a normal patient not having said disorder.

17. A method for preparing PP14.1-specific antibodies, comprising the step of immunizing a host with a polypeptide comprising an antigenic portion of the polypeptide defined by amino acids 33 to 54 of PP14.1.

18. A method for preparing PP14.2-specific antibodies, comprising the step of immunizing a host with a polypeptide comprising a sequence of amino acids overlapping the junctional site of amino acids 32-33 of PP14.2.

19. An antibody specific for PP14.1.

20. A pharmaceutical composition consisting essentially of an antigenic portion of the polypeptide defined by amino acids 33 to 54 of PP14.1.

21. An antibody specific for PP14.2.

22. A portion of a PP14 polypeptide which competitively inhibits the binding of a native PP14 polypeptide to its receptor and has no immunosuppression activity.

23. An anti-idiotypic antibody mimic of PP14 which competes for binding to, but does not activate, a cellular receptor for PP14.

24. A soluble polypeptide derivative of a receptor for PP14 comprising the extracellular domain of a PP14 receptor free of the native transmembrane and cytoplasmic domains of said receptor.

25. A PP14-binding portion of an extracellular domain of a receptor for PP14 linked to a polypeptide tag.

26. Method for treating a patient in need of immunosuppression, comprising the step of administering to said patient a PP14.2 polypeptide.

27. Method for preparing a PP14 polypeptide, comprising the step of isolating PP14 from a hematopoietic cell.

28. The method of claim 27 wherein said hematopoietic cell is a phorbol myristate acetate-induced cell.

29. The method of claim 27 wherein said cell is a platelet.

30. The method of claim 27 wherein said PP14 polypeptide comprises PP14.1.

31. The method of claim 27 wherein said PP14 polypeptide comprises PP14.2.

32. Method for preparing a PP14.2 polypeptide comprising the step of introducing a transcriptional cassette comprising a promoter transcriptionally linked to a portion of a coding sequence for PP14.2.

33. A transcriptional cassette comprising a promoter linked to a portion of a coding sequence for PP14.2.

34. A PP14 receptor-binding portion of a PP14 polypeptide linked to a polypeptide moiety which targets said portion to the surface of an adherent cell.

35. Method for producing a PP14 polypeptide, comprising the steps of:

(a) transfecting a cell with a transcriptional cassette comprising a portion of a PP14 polypeptide linked to a polypeptide moiety which targets said portion to the surface of an adherent cell, to express a PP14 polypeptide linked to said moiety at the cell surface; and

(b) cleaving said moiety with a cleaving reagent, thereby releasing said PP14 polypeptide into the medium.

36. Composition comprising a portion of a PP14 receptor-binding PP14 polypeptide linked to a portion of streptavidin or avidin comprising a biotin-binding domain.

37. Cell comprising a transcriptional cassette comprising nucleic acid encoding a portion of a PP14 polypeptide linked to a polypeptide moiety which targets said portion to the surface of an adherent cell, to express a PP14 polypeptide at the cell surface.

38. Purified heteromultimer comprising PP14.1 and PP14.2 polypeptide chains.

39. Purified homomultimer comprising PP14.2 polypeptide chains.

40. Method for producing a heteromultimer comprising PP14.1 and PP14.2 polypeptide chains comprising the step of cotransfecting PP14.1 and PP14.2 transcriptional cassettes into a cell.

41. A purified or recombinant PP14 receptor.

42. A purified or recombinant PP14 receptor antibody.

43. Purified anti-idiotypic antibody able to bind a PP14 receptor.

44. Method for suppressing natural killer cell activity comprising contacting a natural killer cell with PP14.2.

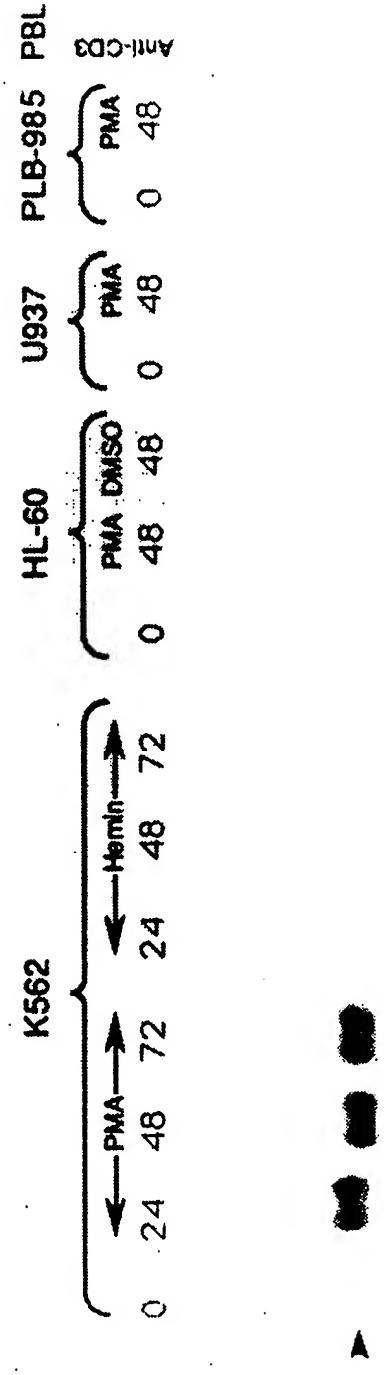
45. A PP14 polypeptide covalently bonded with a polypeptide moiety which targets said PP14 polypeptide to the surface of a cell.

46. The PP14 polypeptide of claim 45 wherein said polypeptide moiety is a cell surface targeting portion of GPI.

47. The PP14 polypeptide of claim 45 wherein said cell is selected from an antigen-presenting cell.

48. Method for coating an antigen-presenting cell with a PP14 polypeptide, comprising the step of providing a PP14 polypeptide adapted to target the surface of said cell.

FIG. 1.



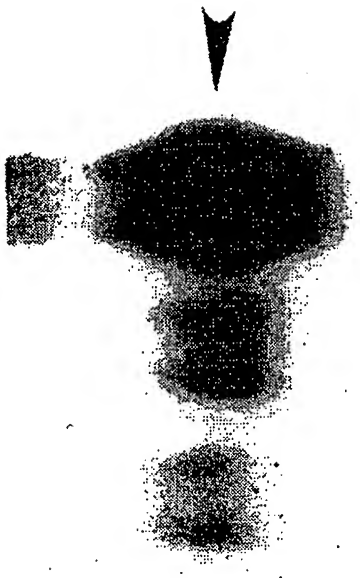
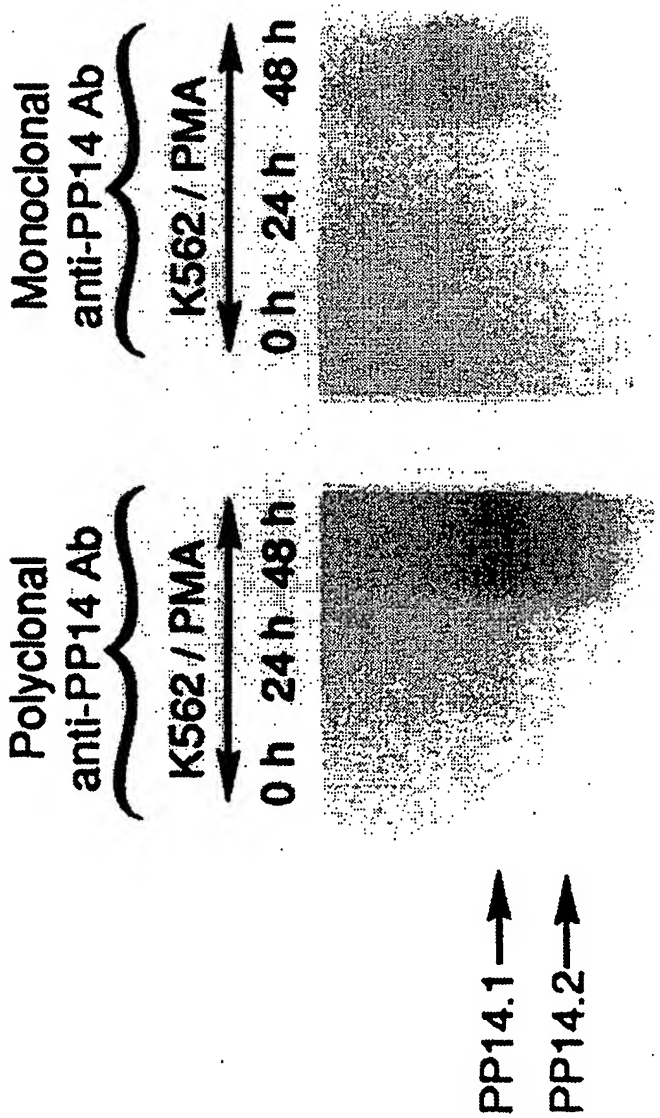


FIG. 2.

FIG. 3.



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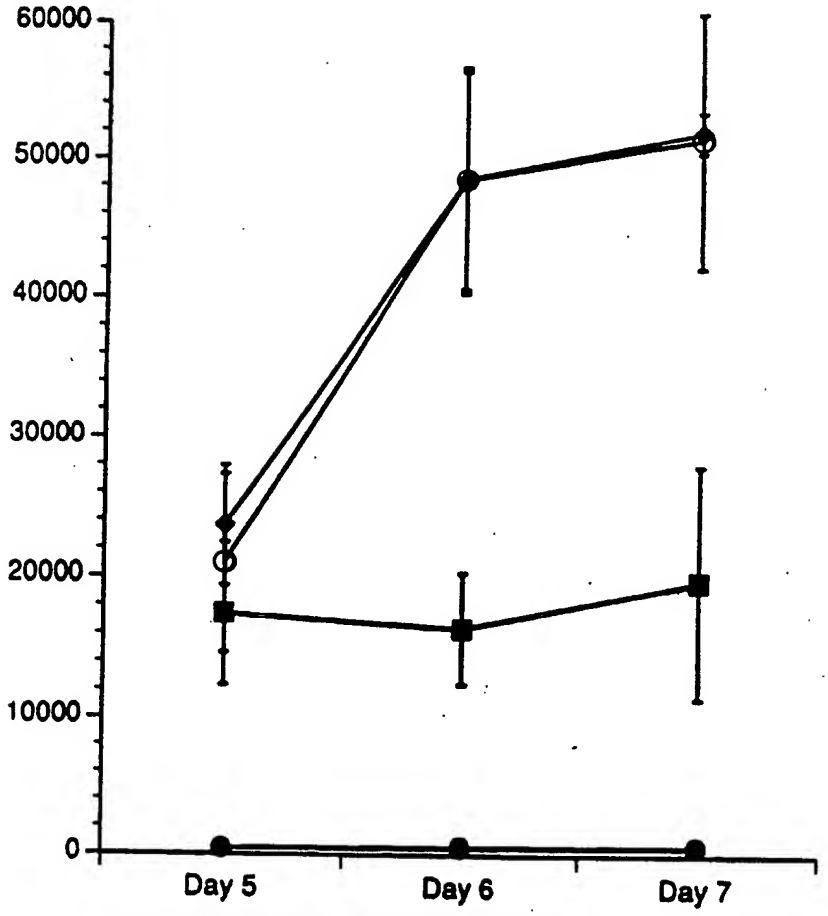

60000
50000
40000
30000
20000
10000
0
Day 5
Day 6
Day 7
Single Donor Only
MLC + uninduced K562 C.M.
MLC + 24h induced K562 C.M.
MLC + 24h induced K562 C.M. + anti-PP14 Ab

FIG. 5a.

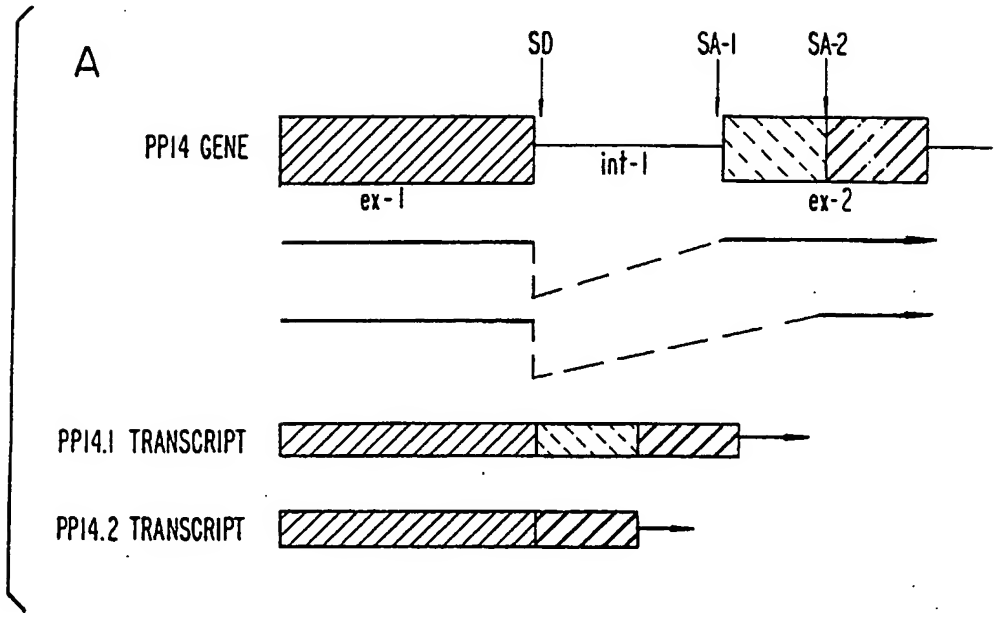


FIG. 5b.

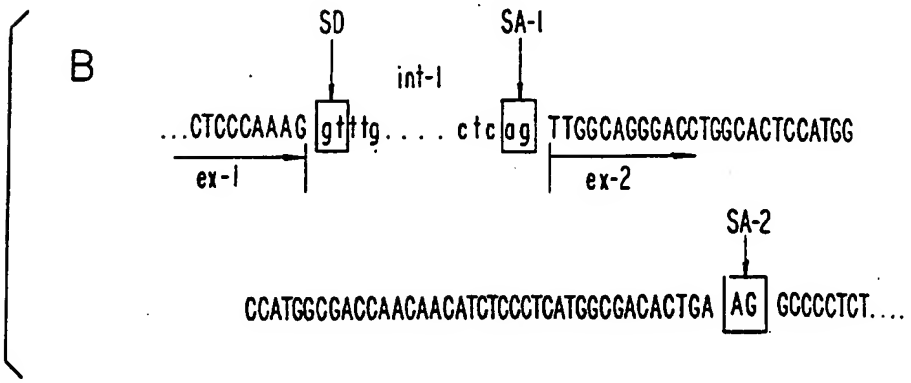


FIG. 5c.

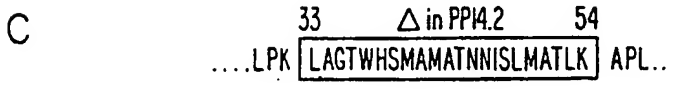
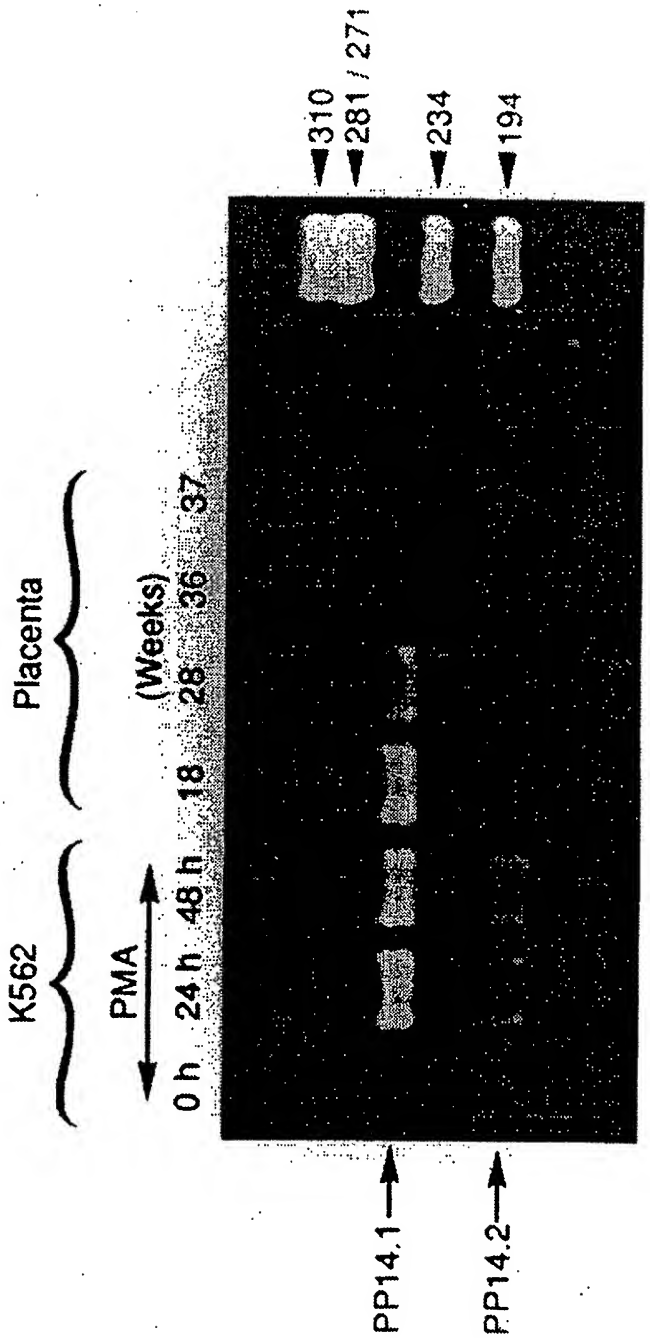


FIG. 6.



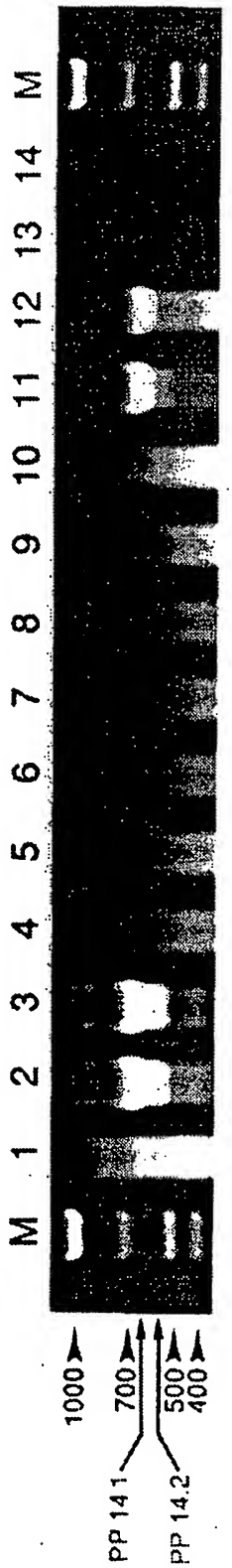


FIG. 7.

INTERNATIONAL SEARCH REPORT

Int. ..ational application No.
PCT/US93/09216

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/85.8; 435/6, 7.1, 69.1, 252.3, 320.1; 514/2, 12, 13, 44; 530/350, 367, 387.2, 389.1, 402, 851; 935/19

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, BIOSIS, CA, INPADOC, JICST-E, MEDLINE, search terms: placental protein 14, isoform, isotype, antibody, dimer, platelet, transcription, DNA, cDNA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US, A, 5,039,521 (Bolton et al) 13 August 1991, col. 4, lines 1-65.	27, 30 1-3,8,9,20, 26,28,29,31-33,35,36
Y	DNA AND CELL BIOLOGY, Volume 9, No. 6, issued 1990, C. Vaisse et al, "Human Placental Protein 14 Gene: Sequence and Characterization of a Short Duplication", pages 401-413, see page 402, column 2, paragraphs 1-2, page 403, column 2, paragraphs 3-5, page 408, Figure 3.	32,33,35,40

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 06 December 1993	Date of mailing of the international search report 20 DEC 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer STEPHEN WALSH Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/09216

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	JOURNAL OF IMMUNOLOGICAL METHODS, Volume 136, issued 1991, L. Riittinen et al, "Monoclonal antibodies against endometrial protein PP14 and their use for purification and radioimmunoassay of PP14", pages 85-90, see page 86, paragraph bridging columns 1-2.	17,19 2,18,21,23
Y	THE LANCET, issued 14 March 1987, A. E. Bolton et al, "Identification Of Placental Protein 14 As An Immunosuppressive Factor In Human Reproduction", pages 593-595, see page 593, summary.	26
A	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, Volume 85, issued December 1988, M. Julkunen et al, "Complete amino acid sequence of human placental protein 14: A progesterone-regulated uterine protein homologous to β-lactoglobulins", pages 8845-8849, see page 8845, abstract.	1-48

INTERNATIONAL SEARCH REPORT

Int...ational application No.
PCT/US93/09216

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
(Telephone Practice)
Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Inte..ational application No.
PCT/US93/09216

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

A01N 43/04; A61K 31/70, 37/02, 39/395, 48/00; C07K 7/10, 15/28; C12N 1/21, 15/10, 15/12; C12P 21/02; C12Q 1/68; G01N 33/53

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/85.8; 435/6, 7.1, 69.1, 252.3, 320.1; 514/2, 12, 13, 44; 530/350, 367, 387.2, 389.1, 402, 851; 935/19

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

I. Claims 1, 2, 6-9, 12, 17-19, 21, 23, 42 and 43, directed to a method of treating immunosuppression by administration of the first named species: antibody, to antibodies and to methods of making antibodies, classified in Class 424, subclass 85.8.

II. Claims 3, 20, 22, 26-40 and 44-48 drawn to a method of treating immunosuppression by administration of the second named species: PP14 peptide, and to a recombinant method of making the peptide, classified in Class 435, subclass 69.1.

III. Claims 4, 5, 10, 11, 24, 25 and 41, directed to a method of treating immunosuppression by adminstration of the third named species: PP14 receptor, to PP14 receptor and to cloning the receptor, classified in Class 435, subclass 69.1.

IV. Claims 13-15, directed to a method of treatment using a reagent that blocks transcription and/or translation, and to a method for identifying a reagent that blocks transcription and/or translation, classified in Class 514, subclass 44.

V. Claim 16, directed to a diagnostic method for identifying a patient with a platelet disorder, classified in Class 435, subclass 7.1.